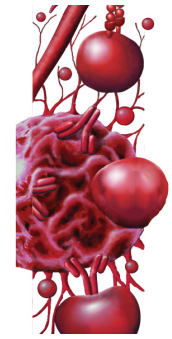
Editorial

Immunotherapy



Chronic Chagas disease: can prophylaxis and therapeutic vaccines crack this 'hard nut'?

"Although, none of the therapeutic vaccines assessed so far have reached sterilizing immunity, the results obtained in terms of disease control indicate that immune intervention might be used for therapeutic purposes."

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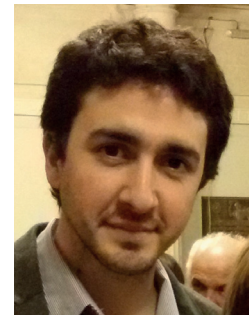
American trypanosomiasis (Chagas disease) is one of the major health problems in Latin America and has emerged as worldwide health problem in the last few years [1]. Mostly, all individuals who are infected by the protozoan *Trypanosoma cruzi* develop an asymptomatic acute phase; with only 1% of them experiencing nonspecific symptoms. After a variable time, about 20–30% of infected people progress to chronic Chagas disease (CCD), which is characterized by mega-syndromes involving the heart and/or the digestive tract. These conditions are responsible for the disabilities and deaths seen among such infected individuals [2].

The underlying mechanisms for CCD imply parasite persistence with the accompanying inflammatory process, and the autoimmune response induced by *T. cruzi* [3]. Also, it has been reported that CCD might be a consequence of functional impairment of pathogen-specific T cells [4]. Despite progress in understanding the pathogenesis of CDD, we still cannot fully explain and predict why and who among the infected individuals will eventually develop symptomatic CCD.

Concerning the treatment, two molecules are currently available as anti-trypanosomal drugs: benznidazole and nifurtimox. Both have shown good efficacy during the acute phase and in the early chronic phase of CCD in children up to 15 years old (level of evidence from meta-analysis of randomized controlled trials) [5]. Nevertheless, as mentioned above, the acute phase is mostly asymptomatic and hence most of the infected individuals who will progress toward chronicity are not diagnosed. In this regard, other important questions are still unanswered; should I treat patients with CCD?, Is the anti-trypanosomal treatment effective to 'cure' individuals with CCD?

On the assumption that reduction in the parasite persistence (and the coexistent inflammatory process) by anti-trypanosomal drugs (including posconazole) might improve the clinical course of CCD, several clinical trials were performed. Unfortunately, these trials and systematic reviews on these studies failed to provide clear favorable outcomes [5,6]. Since none of the studies evaluated hard endpoints and the follow-up was short (up to 4 years), the BENEFIT Trial was carried out. This multicenter, randomized controlled clinical trial was designed to analyze whether benznidazole treatment is able to improve the clinical profile of individuals with Chagas' cardiomyopathy, and to promote confirmation of negativization by PCR. This represented the first study with a considerable sample size (n = 2854) and a longer follow-up (median of 5 years). Despite higher rate of PCR-negative conversion in the benznidazole group, there were no difference in the primary outcomes



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(cardiovascular events) between placebo and benznidazole-treated groups [7]. Recently, the TRAENA trial, that aimed at assessing whether benznidazole modifies the natural evolution of asymptomatic CCD, has been concluded . The results of this study are awaited.

According to available data, there seems to be no benefit in treating CCD patients to elude disease progression. However, beside the limitations of the different studies, the lack of efficacy of benznidazole to prevent progression toward CCD could be due to inadequate treatment as suggested by the BENEFIT report. We should bear in mind that CCD might be independent of parasite persistence and that another physiopathological mechanism, that is, autoimmune response triggered by the protozoan might elicit CCD [3].

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In addition to pharmacological approach, several research groups explored the usefulness of vaccines for CCD. These explorations have been hindered, mainly due to the assumption that vaccines could trigger a severe autoimmune response. Conversely, many reports concluded that specific parasite antigens could be used without inducing autoimmune reaction [8]. In experimental animal models, although none of them were shown to be effective in preventing the infection, they were able to control the disease. Based on these results, the current vaccine research lines are currently oriented on three different aspects: design new vaccines to achieve prevention of the infection; obtain therapeutic vaccines to avoid or delay the involvement of heart and/or digestive tract in individuals with asymptomatic CCD; and obtain therapeutic vaccines to reverse tissue lesions.

Over the years, several *T. cruzi* vaccines employing diverse approaches were developed. These strategies include live attenuated or killed parasite immunization, purified protein immunization, recombinant protein immunization and DNA, bacteria or virus heterologous delivery antigen vaccines [8]. From the vast amount of antigens evaluated, promising results were obtained by CZ, TC24, TcG2, TcG4 and several trans-sialidase family proteins such as TS, ASP2 and TSA-1. As IFN-γ has a protective role in Chagas disease, naked DNA vaccines and viral or bacterial vectors have been employed for the vaccination purposes to boost Th1 response [9]. These approaches have yielded promising results in terms of reducing the parasitemia, cardiac inflammation and parasite burden. These vaccines also tended to reduce the development of cardiac arrhythmias in the chronic stage. Furthermore, recent data showed a long-term efficacy of a prime-boost vaccine, capable of responding to challenge infection 4–6 months post immunization in mice [10]. Similarly, a recombinant human adenovirus vaccine conferred immune protection to the challenge of 70 days after the first prime immunization [11]. However, live recombinant virus or genetic immunization approaches have to pass numerous safety tests that will take many years or decades. Fortunately, researchers are now aware of this urgent requirement of safer vaccines for Chagas disease. For example, Dumonteil E *et al.*, who extensively worked on DNA vaccine for Chagas disease [12], are currently focusing on attempts to reproduce the effect of DNA vaccines by immunizing with their recombinant protein counterparts formulated with alum adjuvant together with the TLR4 agonist [13]. Some authors have already developed subunit vaccines using recombinant proteins formulated with adjuvants of new generations to induce both humoral and cellular immune responses and protection during chronic stage of *T. cruzi* infection, that is, less parasitemia, lower parasite load and fibrotic tissue [14–16]. As $CD4^+CD25^+FoxP3^+$ Tregs influence the clinical course of the disease, adjuvant molecules such as small molecule CCR4 antagonists that transiently inhibit the Tregs during immunization could be explored for the vaccination to boost protective cellular and humoral responses [17].

To date, promising results have been achieved in the acute phase experimental infections in mice and in the prevention of symptoms of chronic disease. These results allow us to think that although prophylactic vaccines may not provide sterilizing immunity, they might be used to prevent the disease**.** Indeed this vaccine approach is being already used in other infectious diseases such as acellular pertussis vaccine. This vaccine has been widely used to prevent whooping cough and severe symptoms of the infection with high efficacy. However, it is now known that it fails to prevent colonization of the bacteria [18]. Finally, several labs are evaluating the therapeutic vaccines in chronic infection models [10–11,13,19]. These vaccines not only prevented but also reversed the clinical and pathological lesions as assessed by ECG, levels of transaminases or histology both in mice and dogs infected with *T. cruzi*. These beneficial effects have been correlated with a decrease in *T. cruzi* persistence in blood and tissue, a lower inflammatory and antioxidant state and anti-inflammatory cytokine profile. Although, none of the therapeutic vaccines assessed so far have reached sterilizing immunity, the results obtained in terms of disease control indicate that immune intervention might be

used for therapeutic purposes. This approach is widely investigated in HIV vaccine research, field where sterilizing immunity seems to be a chimera but where low levels of viral load have shown to avoid AIDS and virus contagion [20].

Given this background, the only effective and available weapon continues to be vector control, which was shown to decrease the incidence and prevalence of Chagas disease in endemic areas. After more than a hundred years of Carlos Chagas first description, we are still awaiting acceptable and successful therapeutic control measures for CCD. Immunotherapeutic has been considered as a possible approach over the last two decades and preclinical results are promising. Though we might get success in the future, for the time being CCD remains 'a hard nut to crack'.

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